

# Starch Biosynthesis

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## INTRODUCTION

Starch is the most significant form of carbon reserve in plants in terms of the amount made, the universality of its distribution among different plant species, and its commercial importance. It consists of different glucose polymers arranged into a three-dimensional, semicrystalline structure—the starch granule. The biosynthesis of starch involves not only the production of the composite glucans but also their arrangement into an organized form within the starch granule. The formation of the starch granule can be viewed as a simple model for the formation of ordered three-dimensional polysaccharide structures in plants. Understanding the biochemical basis for the assembly of the granule could provide a conceptual basis for understanding other higher order biosynthetic systems such as cellulose biosynthesis (see Delmer and Amor, 1995, this issue). For example, one emerging concept is that structure within the granule itself may determine or influence the way in which starch polymers are synthesized.

Starch is synthesized in leaves during the day from photosynthetically fixed carbon and is mobilized at night. It is also synthesized transiently in other organs, such as meristems and root cap cells, but its major site of accumulation is in storage organs, including seeds, fruits, tubers, and storage roots. Almost all structural studies have used starch from storage organs because it is readily available and commercially important; we therefore focus on starch biosynthesis in storage organs. However, where aspects of transient biosynthesis are clearly different from long-term reserve synthesis, reference is made to biosynthesis in nonstorage tissues.

Starch is synthesized in plastids, which in storage organs committed primarily to starch production are called amyloplasts. These develop directly from proplastids and have little internal lamellar structure. Starch may also be synthesized in plastids that have other specialized functions, such as chloroplasts (photosynthetic carbon fixation), plastids of oilseed (fatty acid biosynthesis), and chromoplasts of roots such as carrot (carotenoid biosynthesis). In some cases, for example, in the storage cotyledons of some legumes, amyloplasts in storage organs develop from chloroplasts. These amyloplasts may maintain considerable amounts of stacked lamellar material from the thylakoids and, in cells receiving sufficient light, may undertake some photosynthetic carbon fixation for use in starch

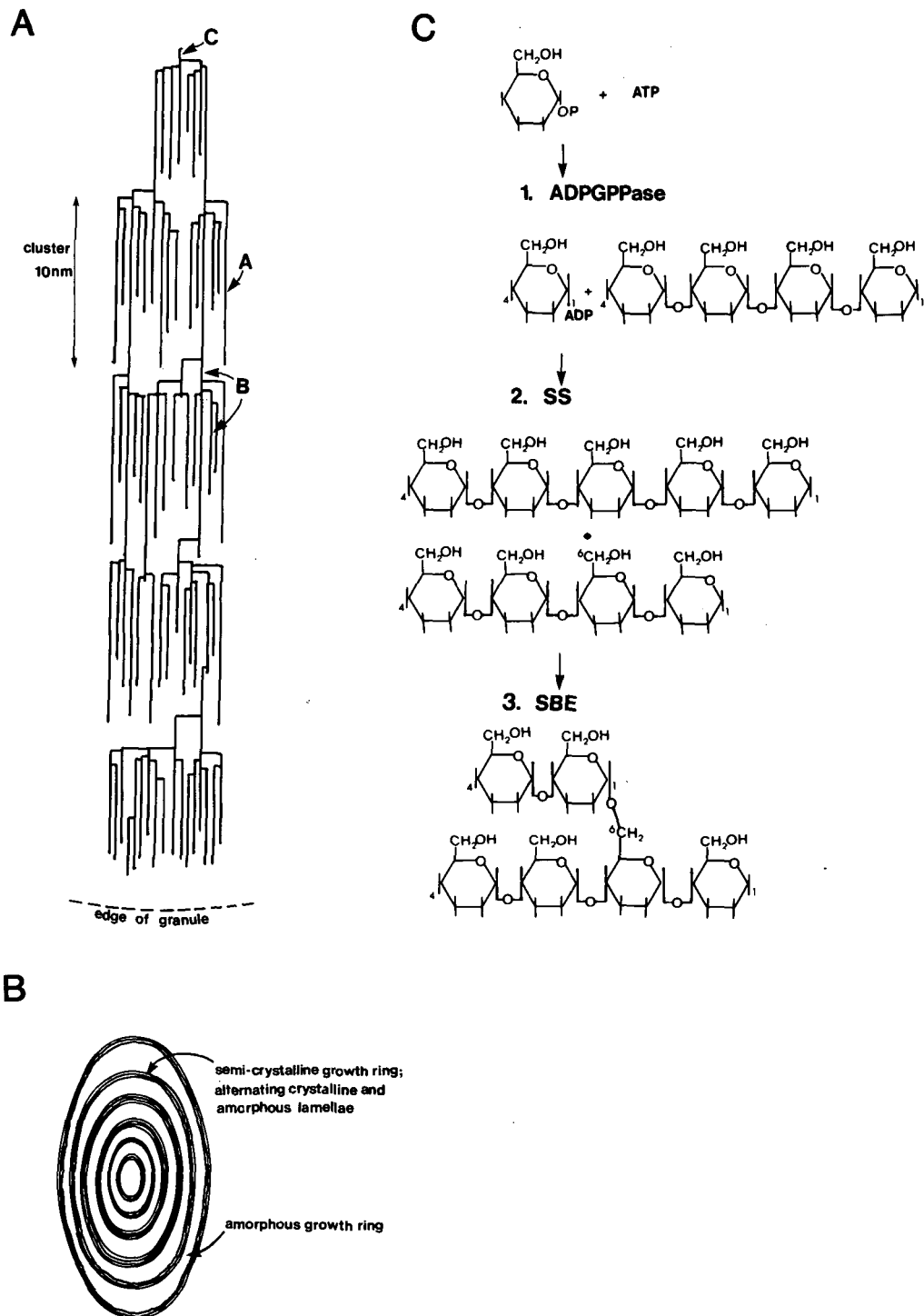
biosynthesis and thus supplement the sucrose imported for starch biosynthesis from the rest of the plant (Smith and Denyer, 1992).

## THE STRUCTURE OF STARCH AND THE STARCH GRANULE

Starch can be chemically fractionated into two types of glucan polymer: amylose and amylopectin. Amylose consists of predominantly linear chains of  $\alpha(1-4)$ -linked glucose residues, each  $\sim 1000$  residues long. Amylose is usually branched at a low level (approximately one branch per 1000 residues) by  $\alpha(1-6)$  linkages and makes up  $\sim 30\%$  of starch. This proportion, however, may vary considerably with the plant species (a range of 11 to 35% was found in a survey of 51 species; Detherage et al., 1955) and variety (a range of 20 to 36% was found in a survey of 399 maize varieties; Detherage et al., 1955) and also with the plant organ, the developmental age of that organ, and, to some extent, the growth conditions of the plant (Shannon and Garwood, 1984). Once extracted from plants and in solution, amylose forms hydrogen bonds between molecules, resulting in rigid gels. However, depending on the concentration, degree of polymerization, and temperature, it may crystallize and shrink (retrogradation) after heating (Shewmaker and Stalker, 1992).

Amylopectin, which consists of highly branched glucan chains, makes up  $\sim 70\%$  of starch. Chains of roughly 20  $\alpha(1-4)$ -linked glucose residues are joined by  $\alpha(1-6)$  linkages to other branches. The branches themselves form an organized structure (Figure 1A). Some are not substituted on the six positions and are called A chains. These chains are  $\alpha(1-6)$  linked to inner branches (B chains), which may be branched at one or several points. A single chain per amylopectin molecule has a free reducing end (the C chain). The branches are not randomly arranged but are clustered at 7- to 10-nm intervals (Figure 1). An average amylopectin molecule is 200 to 400 nm long (20 to 40 clusters) and  $\sim 15$  nm wide (for review, see Kainuma, 1988; Smith and Martin, 1993). After extraction, amylopectin has more limited hydrogen bonding than amylose in solution and is more stable, remaining fluid and giving high viscosity and elasticity to pastes and thickeners. Some

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**Figure 1.** Amylopectin Structure, Starch Granule Form, and Starch Biosynthesis.

**(A)** Diagrammatic representation of an amylopectin molecule.  $\alpha(1-4)$ -Linked glucans are attached by  $\alpha(1-6)$  linkages to form a highly branched structure. Short glucan chains (A chains) are unbranched but linked to multiple branched B chains. There is a single reducing end to the C chain glucan. The branches are arranged in clusters  $\sim 10$  nm long, with a few longer chains linking more highly branched areas.

**(B)** Diagrammatic representation of a starch granule from storage tissue showing alternating semicrystalline and amorphous growth rings. The semicrystalline regions are thought to consist of alternating crystalline and amorphous lamellae.

**(C)** Steps of starch biosynthesis. ADPGPPase catalyzes the formation of ADPglucose and inorganic pyrophosphate from glucose-1-phosphate and ATP (step 1). Starch synthases (SS) add glucose units from ADPglucose to the nonreducing end of a growing  $\alpha(1-4)$ -linked glucan chain by an  $\alpha(1-4)$  linkage and release ADP (step 2). Starch-branching enzymes (SBE) cut an  $\alpha(1-4)$ -linked glucan chain and form an  $\alpha(1-6)$  linkage between the reducing end of the cut chain and the C6 of another glucose residue in an  $\alpha(1-4)$ -linked chain, thus creating a branch (step 3).

starch, most notably that from potato tuber, is also phosphorylated. Potato tuber amylopectin carries up to one phosphate per 300 glucan residues (Takeda and Hizukuri, 1982).

Within the starch granule, which may vary in size from  $<1\ \mu\text{m}$  to  $>100\ \mu\text{m}$ , the amylopectin molecules are arranged radially, and adjacent branches within the branch clusters may form double helices that can be packed regularly, giving a crystallinity to the starch granule. The degree of crystallinity is determined in part by the branch lengths in the amylopectin. Closely packed, highly crystalline structures characteristic of wheat and barley endosperms are associated with average branch lengths of 20 residues. More open structures containing more water are found in potato tuber and are associated with longer branch lengths of 22 glucan residues (for review, see Smith and Martin, 1993). The degree of branching and consequently the crystallinity of starch granules may vary considerably, even between different organs of the same plant. The starch granule is not uniformly crystalline, however, and contains relatively amorphous regions. Amylose molecules form single helical structures and are thought to be packed into these amorphous regions, which are present throughout the granule.

Granules from storage organs and leaves have rather different macrostructures. Starch granules from storage organs show internal semicrystalline growth rings that are differentially sensitive to chemical and enzymatic attack (Figure 1B). The denser, more resistant layers may be regions of closer packing of branches within the branch clusters of parallel amylopectin molecules. The formation of these rings may result from periodic differences in the rate of starch synthesis. For example, in wheat endosperm the growth rings follow clear diurnal patterns and are lost in plants grown under continuous illumination (Buttrose, 1962; French, 1984). However, growth rings are not universally responsive to illumination and may result from other periodic fluctuations in biosynthesis.

Starch granules in leaves are generally smaller than those in storage organs and have a distinct macrostructure. They are thought to have a crystalline core with an amorphous outer mantle that consists of less highly branched glucan polymers (Steup et al., 1983; Beck, 1985). Most of the turnover in starch during day/night cycles involves the amorphous mantle of the granules (Steup et al., 1983; Beck, 1985).

Other components within all starch granules are proteins (0.5% in cereal endosperm and 0.05% in potato tuber), which include the enzymes of starch biosynthesis and may contribute to the flavor of starch, and lipid (1% in cereal endosperm and 0.1% in potato tuber). The chemical structure of amylose facilitates its association with lipid, so the lipid may be localized to specific regions within the starch granule.

## THE BIOCHEMISTRY OF STARCH BIOSYNTHESIS

The biosynthetic steps required for starch biosynthesis are relatively simple, involving three committed enzymes: ADP-

glucose pyrophosphorylase (ADPGPPase; EC 2.7.7.23), starch synthase (SS; EC 2.4.1.21), and starch branching enzyme (SBE; EC 2.4.1.28; Figure 1C).

Both amylose and amylopectin are synthesized from ADP-glucose, which is synthesized from glucose-1-phosphate and ATP in a reaction that is catalyzed by ADPGPPase and that liberates pyrophosphate. This enzyme is active within the plastid, which means that its substrates, glucose-1-phosphate and ATP, must also be present in the plastid. In chloroplasts, ATP may be derived from photosynthesis, but in nonphotosynthetic plastids, it must be specifically imported from the cytosol, probably by an ADP/ATP translocator (Ngernprasirtsiri et al., 1989; Schünemann et al., 1993). The glucose-1-phosphate can be supplied by the reductive pentose phosphate pathway in chloroplasts via phosphoglucisomerase and phosphoglucomutase (Smith and Martin, 1993). In nonphotosynthetic tissues, it may be imported directly from the cytosol (Tyson and ap Rees, 1988) or synthesized in the plastid from glucose-6-phosphate via the action of a plastidial phosphoglucomutase (Hill and Smith, 1991).

The pyrophosphate produced by ADPGPPase is removed by inorganic alkaline pyrophosphatase, which is probably confined to plastids in both photosynthetic and nonphotosynthetic tissues. The removal of this plastidial pyrophosphate effectively displaces the equilibrium of the ADPGPPase reaction in favor of ADPglucose synthesis (Weiner et al., 1987).

In the next step of starch synthesis, SS catalyzes the synthesis of an  $\alpha(1-4)$  linkage between the nonreducing end of a preexisting glucan chain and the glucosyl moiety of ADP-glucose, causing the release of ADP. Ss can use both amylose and amylopectin as substrates *in vitro*. How the initial primers for the synthesis of glucan chains are produced *in vivo* is not known. There is some biochemical evidence for specialized activities that may build protein-glucose or protein-polyglucose primers in potato tubers. One report suggested that UDPglucose is used as a precursor to transglycosylate an acceptor protein (Moreno et al., 1986). However, UDPglucose levels are believed to be very low in plastids, leaving the importance of such a priming system open to question. In bacteria, which synthesize glycogen via a pathway that has many similarities with starch synthesis in plants, a potential priming protein has been identified genetically (Hengge-Aronis and Fisher, 1992).

The  $\alpha(1-6)$  branches in starch polymers are made by SBE, which hydrolyzes an  $\alpha(1-4)$  linkage within a chain and then catalyzes the formation of an  $\alpha(1-6)$  linkage between the reducing end of the "cut" glucan chain and another glucose residue, probably one from the hydrolyzed chain. Branches are not created randomly, as discussed previously, but show an average periodicity of 20 glucan residues. SBEs show some specificity for the length of the  $\alpha(1-4)$  glucan chain that they will use as a substrate. Part of this selectivity may reside in the fact that these enzymes cleave only those glucan chains that are in a stable double helical conformation, a structure that requires a minimum glucan chain length.

## VARIABLE PARAMETERS IN STARCH BIOSYNTHESIS

The relative simplicity of the starch biosynthetic pathway does not explain the enormous variability in starch composition among different plant species, varieties, and tissues. Nor does it explain the complexity of starch in terms of its component glucan chains and their organized arrangement in starch granules. We are only just beginning to understand how these layers of complexity are determined, but already it is clear that central to their organization is diversification in the activities of the participating enzymes and modulation of the extent of their activities.

In all species that have been investigated, there are isoforms for each of the committed steps of starch biosynthesis. These may differ in their products, their kinetic properties, their time of expression during starch granule formation, and the organs in which they are active. The existence of isoforms clearly provides flexibility for specialization and control in starch biosynthesis. One problem in characterizing isoforms for each step has been that their identification has been based predominantly on activities in biochemically fractionated extracts. This approach has allowed many proteins with either SS or SBE activity to be characterized from different species. However, it is unlikely that these are all the products of different genes, because protein degradation is a common feature of purification of SSs and SBEs (see, for example, Blennow and Johansson, 1991; Baba et al., 1993; Mu et al., 1994) and could indeed be of significance *in vivo*. The understanding of the roles of different isoforms is therefore being greatly facilitated by molecular analysis, which has begun to allow the assignment of isoforms to particular families with related primary structures and apparently related functions. These assignments allow not only the comparison of the roles of particular isoforms from different species but also a clearer view of how starch biosynthetic gene expression is controlled and the contribution this control makes to the overall regulation of starch biosynthesis.

Some modulation of starch biosynthesis can also be achieved through metabolic control of flux through the pathway. In leaves, starch synthesis occurs at higher rates when carbon assimilation is high relative to the demand for carbon export and at lower rates when assimilation is low relative to demand from the rest of the plant. There is strong evidence that changes in rate are achieved through allosteric regulation of ADPGPPase by the activator 3-phosphoglycerate (3-PGA) and the inhibitor inorganic phosphate (Pi). Changes in the levels of 3-PGA and Pi in leaves are modulated primarily by the rate of photosynthetic carbon fixation, thus giving rise to significant modulation of ADPGPPase activity (Preiss, 1991). It is possible that the contribution of metabolic regulation to starch biosynthesis may vary across the plant. Starch biosynthesis in many storage organs does not have an obvious requirement for short-term metabolite-mediated regulation, and several reports indicate that the potential for allosteric regulation of ADPGPPase from some storage organs is relatively

insignificant in comparison with that in leaves (Hylton and Smith, 1992; Kleczkowski et al., 1993; Weber et al., 1995). Consequently, control of starch biosynthesis may also involve modulation of the extent of allosteric regulation of ADPGPPase. Of course, the activity of ADPGPPase may exercise significant control in starch biosynthesis, even in tissues in which allosteric regulation is not important; however, this needs to be tested empirically in different tissues and under different conditions.

The different proteins involved in starch biosynthesis may also vary in their physical characteristics, which can have a profound effect on the products made within the starch granule. This is most clearly seen for SSs, which are located both bound to the starch granule and in the soluble phase of the amyloplast. Following biochemical analysis of waxy (*wx*) mutants from several species, a functional distinction was predicted, namely, that granule-bound SSs (GBSSs) would synthesize amylose, whereas soluble SSs would synthesize amylopectin. More recent analysis of SSs has shown that the biochemical distinctions are not absolute; SSs found in the soluble phase may also be bound to the granule (Denyer et al., 1993, 1995). However, "noncatalytic" characteristics are probably still of functional significance because they could dictate how active a particular isoform may be when bound to the granule. To date, most assays of starch biosynthetic enzymes have been made on soluble or solubilized extracts, which may not reflect precisely the conditions within the granule. Therefore, it is difficult to assess the importance of such "noncatalytic" properties without undertaking lengthy *in vivo* assays using mutagenesis and plant transformation. However, the presence of particular noncatalytic characteristics in different forms of each biosynthetic enzyme potentially represents another way in which the pathway can be diversified to give more complexity in glucan products and their organization and more variation among different plant species.

Starch biosynthesis varies both quantitatively and qualitatively during the course of storage organ formation. Some isoforms of the biosynthetic enzymes may be active early in starch granule formation and others active later. For example, amylose content normally increases as a proportion of total starch during storage organ development, indicating that its synthesis is somewhat delayed compared with that of amylopectin (Shannon and Garwood, 1984). This is likely due partly to the timing of production of the amylose-specific GBSS, which is synthesized later than some other SSs (Nelson et al., 1978; Dry et al., 1992).

There may also be developmental gradients in starch biosynthesis within a storage organ (Shannon and Garwood, 1984). For example, waves of gene expression have been reported across developing seed from more advanced to less advanced cells (Shannon and Garwood, 1984; Perez-Grau and Goldberg, 1989; Hauxwell et al., 1990). Whereas some species show significant differences in expression of starch biosynthetic genes at different developmental stages (Dry et al., 1992; Nakamura and Yuki, 1992; Burton et al., 1995), others show few differences (Kossmann et al., 1991; Mizuno et al.,

1993). This apparent lack of developmental change could be an artifact of the way gene expression is assayed; storage organs with strong internal developmental gradients tend to "flatten out" developmental differences in assays based on total extracts. In fact, it may be that developmental regulation of isoform gene expression is more important than is appreciated at present.

A final factor complicates the potential significance of developmental modulation in the control of starch biosynthesis. As the starch granule grows, many of the biosynthetic enzymes become trapped within it (Denyer et al., 1993, 1995). This means that the turnover of these enzymes is relatively low. However, it is unclear to what extent the trapped enzymes are active within the granule; thus, the effective activity of the biosynthetic enzymes at different developmental stages *in vivo* is very difficult to assess.

The plant can thus use several different strategies to refine starch biosynthesis and to build the organized form of the granule. Although our understanding is far from complete, the use of molecular biology and genetics has complemented the biochemical analysis of this system to allow a greater appreciation of the control of each biosynthetic step and its contribution to the overall process.

## THE CONTRIBUTION OF EACH BIOSYNTHETIC STEP TO STARCH BIOSYNTHESIS

### Supply of ADPGlucose Precursors

ADPGPPase supplies ADPGlucose for starch biosynthesis, and under some conditions, its activity is the most significant factor in determining the rate of starch accumulation. This has been established most convincingly in leaves of *Arabidopsis*. A mutation in a gene encoding one of the two subunits of the enzyme reduces ADPGPPase activity to 7% and starch accumulation to 26% of normal values in leaves (Lin et al., 1988). This mutant has been used to estimate the flux control coefficient (Kacser and Burns, 1973) for *Arabidopsis* ADPGPPase, which is calculated from the relationship between the rate of starch synthesis and the ADPGPPase activity in wild-type and mutant leaves (Neuhaus and Stitt, 1990). Under low irradiance, ADPGPPase exercises the most significant degree of control on starch synthesis in leaves. Under high irradiance, although the flux control coefficient for ADPGPPase increases, the coefficients for other enzymes also increase, as deduced by measurements from other species, indicating that control is exercised by additional enzymatic steps. These additional control points are likely to involve enzymes that supply hexose phosphate from photosynthetic carbon fixation. The role of ADPGPPase in controlling starch biosynthesis in storage organs has not been measured in this way.

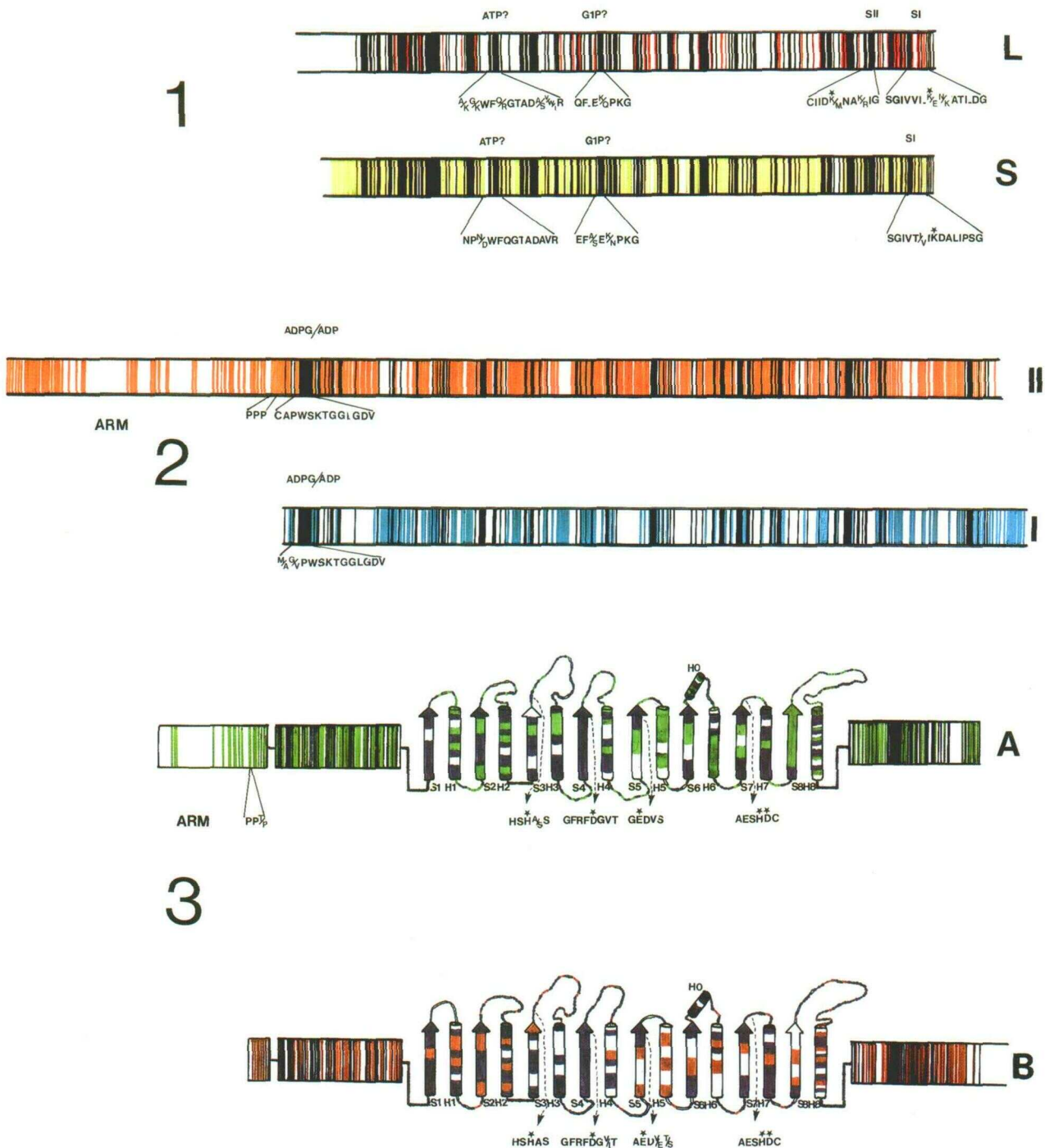
Much significance has been placed on the allosteric regulation of ADPGPPase by 3-PGA and Pi in the control of starch biosynthesis in all organs of the plant (Plaxton and Preiss, 1987;

Preiss, 1991, 1993; Preiss et al., 1991; Okita, 1992). This view is supported by the experiments of Stark et al. (1992), who were able to increase starch biosynthesis in cultured tobacco cells by 300% and in potato tubers by up to 60% by transformation with an *Escherichia coli* gene encoding a version of ADPGPPase that is insensitive to allosteric regulation. The normal *E. coli* ADPGPPase is sensitive to allosteric regulation by fructose-1,6-bisphosphate (Preiss and Romero, 1989), and this regulated enzyme is not able to increase starch production in transgenic tissues, which was interpreted as indicating that it is, indeed, the allosteric regulation of ADPGPPase that is important in determining starch accumulation in cultured tobacco cells and potato tubers (Stark et al., 1992). However, because the regulated *E. coli* enzyme is not under the same metabolic control as the normal plant enzyme, the significance of allosteric regulation would be demonstrated more compellingly by comparing the effects of the nonregulated enzyme with the effects of increased activities of the normal plant enzyme.

Although the significance of allosteric regulation in the control of starch synthesis in storage organs therefore remains under investigation, it is reasonable to conclude from these experiments that ADPGPPase activity does exert some control over starch biosynthesis, at least in light-grown, cultured tobacco cells and potato tubers. Interestingly, however, in these experiments, there was no significant correlation between the degree of activity of the unregulated ADPGPPase and the increase in starch accumulation, indicating that the activity of ADPGPPase may limit starch accumulation under normal conditions but that other steps also exercise significant control.

Plant ADPGPPase is reported to be a tetrameric enzyme that is formed from two distinct polypeptides (Copeland and Preiss, 1981), one between 54 and 60 kD (the large subunit) and one between 51 and 55 kD (the small subunit). cDNA clones encoding both subunits have been isolated from several plant species (Olive et al., 1989; Bae et al., 1990; Bhavé et al., 1990; Müller-Röber et al., 1990; Smith-White and Preiss, 1992; Villand et al., 1992a, 1992b; Weber et al., 1995); their deduced amino acid sequences share regions of homology, and they may in fact have evolved from a common ancestor because the bacterial enzyme is a homotetramer of a polypeptide with a similar amino acid sequence (Inglesias et al., 1991; Smith-White and Preiss, 1992).

Expression of both potato subunits in *E. coli* has demonstrated that each has a low ADPGPPase activity alone but that this activity can be enhanced 10- to 70-fold by mixing them or by coexpressing them. The potato enzyme produced in this way remains responsive to 3-PGA (Inglesias et al., 1993). These and other experiments suggest that the plant enzyme normally functions as a heterotetramer of two large and two small subunits. Pyridoxal phosphate analysis, which can be used to identify the site of 3-PGA binding, has shown that a conserved lysine residue at the C terminus of the small subunit and the equivalent lysine residue in the large subunit bind this 3-PGA analog (Figure 2; Morell et al., 1988; Preiss, 1993). A second lysine residue in the large subunit from spinach leaf also binds pyridoxal phosphate; this residue may respond to



**Figure 2.** Diagrammatic Representation of the Homologies among Starch Biosynthetic Enzymes.

**(1)** Subunits of ADPGPPase. The homologies between the deduced amino acid sequences of the large (L) and small (S) subunits from different species are represented by black bars, which indicate residues conserved between both subunits. Red bars indicate residues conserved among large subunits, and pale green bars indicate residues conserved among the small subunits. The sites showing homology with ATP and glucose-1-phosphate (G1P) binding sites in glycogen synthase from *E. coli* (Preiss, 1993) are indicated, as is the sequence (SI) around the conserved lysine (starred) in the small subunit that binds the allosteric regulator analog pyridoxal phosphate. This is thought to represent a binding site for 3-PGA. The equivalent site (SI) in the large subunit is also indicated, along with a second site (SII) that may be involved in both 3-PGA and Pi regulation

Pi regulation as well (Figure 2; Preiss, 1993). However, this second lysine residue is not conserved within the large subunits from all sources. Most notably, in the large subunits expressed in wheat and barley endosperm it is replaced by methionine, although in the large subunit expressed in wheat leaves it is a lysine residue. This suggests that the degree of regulation exercised by 3-PGA and Pi could vary with the isoform of the large subunit expressed in different parts of the plant.

Analysis of the maize *brittle2* (*bt2*) mutant (which affects the small subunit in the endosperm) and the maize *shrunk2* (*sh2*) mutant (which affects the large subunit in endosperm) has shown that both the large and small subunits are required for full ADPGPPase activity. Both mutations abolish 90 to 95% of ADPGPPase activity in endosperm, giving up to 75% less starch (Tsai and Nelson, 1966; Dickinson and Preiss, 1969; Bae et al., 1990; Bhavé et al., 1990). In pea, mutation of the *rugosus* (*rb*) locus affects a gene encoding the large subunit and results in a 90% or greater reduction of ADPGPPase activity and a concomitant drop of ~50% in starch accumulation (Hylton and Smith, 1992; R.A. Burton, C. Martin, and R. Hellens, unpublished results). The loss of a functional large subunit results in a less active enzyme that is also more sensitive to regulation by 3-PGA and Pi (Smith et al., 1989; Hylton and Smith, 1992). This suggests that in pea embryos, the two subunits of ADPGPPase may make unequal contributions to the allosteric regulation of the enzyme. Li and Preiss (1992) have also reported that mutation of the large subunit in *Arabidopsis* results in a difference in ADPGPPase response to 3-PGA and Pi, although in this case the response is reduced in the mutant line. In general, it may be that different isoforms of the subunits (and, in particular, of the large subunit) confer on the enzyme different degrees of sensitivity to allosteric regulation.

In all plants that have been tested, including *Arabidopsis* (Villand et al., 1993), potato (Müller-Röber et al., 1990), barley (Villand et al., 1992a, 1992b), rice (Krishnan et al., 1986), and wheat (Olive et al., 1989), multiple genes encode the large

subunit. These genes show strong specificity in their expression, for example, being restricted to either leaf or root and endosperm in both barley and wheat (Olive et al., 1989; Villand et al., 1992a, 1992b) or being induced under specific conditions, such as increased sucrose levels in potato (Müller-Röber et al., 1990). Genetic analysis confirms the differential use of large subunit isoforms. Because the effects of the maize *sh2* mutation are confined to the endosperm, another gene(s) must provide the function of the large subunit in embryos and leaves (Shannon and Garwood, 1984). In fact, a second transcript encoding the large subunit in maize embryos has also been shown to accumulate at low levels in endosperm (Giroux and Hannah, 1994).

Similarly, there may be multiple isoforms of the small subunit. Two cDNA clones have been identified from bean that show organ-specific expression patterns: one form is expressed only in leaves, the other in both leaves and cotyledons (Weber et al., 1995). Two different cDNAs encoding the small subunit have also been identified in maize, and both are expressed in distinct tissue-specific patterns (Giroux and Hannah, 1994; Prioul et al., 1994).

The functional significance of the tissue-specific expression of isoforms of both subunits of ADPGPPase awaits further characterization of the activity of each isoform alone and in combination with the other subunit. If, for example, one subunit is expressed constitutively, as Müller-Röber et al. (1990) have suggested is the case in potato, the enzyme would be strongly activated upon expression of the other subunit. In this respect, the association of large and small subunits needs to be investigated to determine whether the protein is always composed of two subunits of each or whether imbalances in the relative availability of the two peptides might instead result in the production of an enzyme with altered subunit composition and consequently altered activity. Variability in ADPGPPase activity might also result if isoforms differ in their ability to bind allosteric regulators. Different combinations of large and small subunits would thus show differential sensitivity to allosteric

Figure 2. (continued).

(Preiss, 1993). Sequences were aligned using the Pileup program (Devereux et al., 1984) from sequences reported by Olive et al. (1989), Bae et al. (1990), Bhavé et al. (1990), Müller-Röber et al. (1990), Smith-White and Preiss (1992), Villand et al. (1992a, 1992b, 1993), Weber et al. (1995), and R.A. Burton and C. Martin (unpublished results).

(2) Starch synthases. The homologies between the deduced amino acid sequences of GBSSII (II) and GBSSI (I) from different species are represented by black bars, which indicate residues conserved between both isoforms. Orange bars indicate residues conserved in GBSSIs and blue bars those conserved in GBSSIs. The conserved ADPglucose/ADP binding sites are indicated, as is the flexible arm of GBSSII. Sequences were aligned using Pileup (Devereux et al., 1984) from sequences referred to in Dry et al. (1992) and obtained by E.A. Edwards and C. Martin (unpublished results).

(3) Starch branching enzymes. The homologies between the deduced amino acid sequences of SBE isoform A (A) and isoform B (B) are represented by black bars, which indicate residues conserved between both families. Dark green bars indicate residues conserved in family A members and brown bars those conserved in family B members. The structure of SBEs has been modeled on the  $(\beta\alpha)_8$  barrel structure of  $\alpha$ -amylases (Jespersen et al., 1993). The eight regions of  $\beta$  strand (S1 to S8) and  $\alpha$  helix (H1 to H8) are indicated. Residues in this region are not drawn to scale. In addition, sequences conserved in the active sites of  $\alpha$ -amylases and other  $\alpha$ (1-4)hydrolases are indicated. The residues of the active site involved in the catalytic mechanism are starred. The N-terminal arm in family A is marked, and the conserved region of additional  $\alpha$  helix is labeled HO. Sequences were aligned by Pileup (Devereux et al., 1984) as referred to by Burton et al. (1995).



regulation. Such differences have been reported for the enzyme isolated from cereal endosperm when compared with that isolated from cereal leaves (Duffus, 1993; Kleczkowski et al., 1993) and for the enzyme from bean cotyledons when compared with that from bean leaves (Weber et al., 1995).

In addition to affecting the amount of starch produced, ADPGPPase appears to influence starch composition. This is suggested by the fact that the *rb* mutation of pea, which affects the large subunit of ADPGPPase, not only reduces the total amount of starch produced but also alters its quality by increasing the proportion of amylopectin (Kooistra, 1962). Analysis of other pea mutants that show reduced starch accumulation through effects on steps involved in the supply of precursors shows that reductions in activity of these enzymes also elevates amylopectin content, suggesting that the effects on composition are a general result of decreased starch synthesis rather than a specific effect of ADPGPPase (T. Wang, personal communication).

Why does a decrease in starch synthesis increase the proportion of amylopectin? One possibility is that SBE is normally limiting for the synthesis of amylopectin, so increased branching is observed when the rate of  $\alpha(1-4)$ glucan synthesis is reduced. If this were the case, then a mutation inhibiting SBE activity should severely affect the production of amylopectin, even in heterozygous condition. Maize endosperm containing two doses of the *amylose extender* (*ae*) mutation, which reduces activity of one SBE isoform, and just one dose of the wild-type allele show a slight (2 to 8%) decrease in the apparent amylopectin content (Shannon and Garwood, 1984). An alternative, but not mutually exclusive, possibility is that reduced levels of ADPglucose may have particularly extreme effects inside the starch granule, where availability is dependent on diffusion through the crystalline matrix. The reduced ADPglucose availability could have a relatively greater effect on the amylose-specific GBSS, which is located exclusively inside the granule, than on the SSs in the soluble phase, which synthesize amylopectin.

### Synthesis of $\alpha(1-4)$ Glucan Chains

Collectively, SSs extend  $\alpha(1-4)$ glucan chains and synthesize both amylose and amylopectin. However, the amylose that they produce in vivo is somehow protected from the activity of SBEs in the plastid, even though SBEs can use it as a substrate in vitro. The key to understanding the synthesis of these discrete products lies in the discovery that *wx* mutants of maize, rice, sorghum, and *Amaranthus*, and the *amylose free* (*amf*) mutant of potato, make no amylose and lack the activity of an exclusively GBSS (GBSSI; Sprague et al., 1943; Nelson and Rines, 1962; Tsai, 1974; Okuno and Sakaguchi, 1982; Sano, 1984; Hsieh, 1988; Jacobsen et al., 1989) that has also been frequently referred to as the "waxy" protein. This ~60-kD protein is made in relatively large amounts and is found exclusively bound to the starch granule. It has a low specific activity in standard assays (Nelson et al., 1978; Macdonald and Preiss, 1985)—so low that in some species it has been impossible

to detect activity in vitro (Smith, 1990; Denyer et al., 1995). However, data from mutants on the effect of the loss of this isoform support the view that it is responsible for the synthesis of amylose. It may be that the standard assays used to measure SS activity do not accurately reflect the ability of this protein to synthesize  $\alpha(1-4)$ glucan chains in vivo because it is specifically localized within the starch granule, where the crystalline matrix of glucan chains may provide a specialized environment that promotes the activity of this isoform.

There is some evidence from the equivalent amylose-free mutant of the alga *Chlamydomonas* that GBSSI may also be responsible for the synthesis of a significant proportion of amylopectin, because the mutant shows deficiencies in a distinct amylopectin fraction as well as lacking amylose (Delrue et al., 1992). In higher plants, mutations that eliminate GBSSI activity do not reduce starch content, implying that the ADPglucose not used to make amylose is used by other SSs to make amylopectin. If GBSSI contributes to amylopectin biosynthesis, this suggests that its ability to make amylose is not an intrinsic property of the protein but rather a function of its location in the starch granule. Perhaps it makes amylose when it is relatively deep within the granule and amylopectin when it is closer to the outer edge of the granule, where its products are accessible to the SBEs, which are active predominantly in the soluble phase. This idea is supported by the observation that in starch granules of potato lines in which GBSSI activity is reduced by antisense suppression, amylose is restricted to a central core region (Kuipers et al., 1994). This could imply that amylose production requires a starch granule structure in which the GBSSI is "buried." If so, this might explain in part why amylose production appears to lag behind that of amylopectin during development of storage organs (Shannon and Garwood, 1984).

Other SS isoforms are located in the soluble phase of the amyloplast, although it is probably more useful to think of these isoforms in terms of the families to which they belong rather than in terms of their classic biochemical separation into soluble and granule-bound forms, because some are clearly located in both phases. One SS from pea embryos is located both in the soluble phase and on the granule and has been called GBSSII, although it is believed to be the major isoform active in the soluble phase in embryos (Smith, 1990; Denyer and Smith, 1992; Dry et al., 1992). Potato tubers contain a homolog of this isoform (Dry et al., 1992), which is found in the soluble phase and is also granule bound; there are probable homologs in maize and wheat (Mu et al., 1994; Denyer et al., 1995). GBSSII has a much higher specific activity than GBSSI when measured in vitro (Smith, 1990; E.A. Edwards, C. Martin, and E. Murray, unpublished results). However, members of other isoform families are certainly active in different parts of the plant and in different species, and the relative contribution of each isoform to  $\alpha(1-4)$ glucan synthesis may vary (Denyer et al., 1995).

GBSSII from pea is structurally similar to GBSSI from maize and potato and to glycogen synthase from bacteria (Dry et al., 1992). However, it contains at its N terminus an extra domain not present in the other two enzymes. This domain accounts



for the greater size of this SS isoform, which is some 17 kD larger than pea GBSSI. The domain is rich in serine and therefore predicted to be highly flexible. Three consecutive proline residues near the C terminus of the extension may specify a turn in the folded protein (Figure 2). These residues are also present in the homologous protein from potato. We have termed this domain the "flexible arm" of GBSSII. Full-length GBSSII expressed in *E. coli* has the same specific activity as the purified plant enzyme, and versions lacking the arm are also fully active, indicating that the arm does not participate in the catalytic mechanism of the enzyme. Antisera specific to pea GBSSII cross-react with the *E. coli*-produced proteins with or without the arm, and antisera specific to GBSSI do not cross-react with either of these proteins, indicating that the structurally similar regions of GBSSI and GBSSII that lie C terminal to the arm (in the case of GBSSII) are antigenically distinct (E.A. Edwards, J. Marshall, A.M. Smith, and C. Martin, unpublished results). This antigenic specificity is maintained toward the homologous proteins from other species—the pea GBSSII antibody recognizes potato GBSSII but not potato GBSSI, for example—implying that there may be significant structural differences between GBSSI and GBSSII isoforms beyond the presence or absence of the N-terminal arm. These differences are likely to determine differences in the specific activities of the enzymes (low for GBSSI, high for GBSSII), whereas the N-terminal domain of GBSSII is more likely to affect noncatalytic properties of the enzyme, such as the potential for physical association with SBEs or its degree of partitioning between soluble and granule-bound phases of the amyloplast.

The only other SS for which there is detailed structural information is a soluble SS from rice endosperm. Sequencing of cDNA clones (Baba et al., 1993) indicates that this protein contains a C-terminal region with strong homology with *E. coli* glycogen synthase and other plant SSs. At its N terminus is a proline-rich region that ends in three consecutive proline residues. The amino acids surrounding this Pro-Pro-Pro motif show some homology with the equivalent region at the end of the arm of GBSSII from pea and potato (Dry et al., 1992). This region in the rice-soluble SS has been interpreted as the transit peptide of the protein (Baba et al., 1993), but it does not conform to typical transit peptides in terms of size, sequence composition, or hydrophobicity, and it is more likely to represent part of a longer N-terminal extension within SS that has certain features in common with GBSSII from pea and potato. The assignment of the end of the mature protein of the rice-soluble SS to a position C-terminal to the potential arm was based on N-terminal sequencing of 57-kD polypeptides prepared from partially purified preparations of rice-soluble SS. These may have been derived from a larger form of SS; indeed, similar potential low molecular weight derivatives of GBSSII have been observed in SS preparations from pea embryos (Denyer and Smith, 1992). These maintain SS activity and therefore probably involve loss of parts of the N-terminal arm that may be particularly sensitive to proteases. Such products probably also arise *in vivo* and may explain reports of a high-specific activity, low-molecular weight GBSS from pea (Sivak et al., 1993). Loss of the arm could result in changes

in the physical properties of the soluble SS, affecting its partitioning between the soluble and granule-bound phases.

In summary, SS isoforms have a conserved C-terminal region of ~60 kD that is similar in sequence to glycogen synthase from bacteria (Figure 2). The plant isoforms that are active in the soluble phase and that have been characterized at the molecular level probably also have N-terminal domains of high predicted flexibility that affect their noncatalytic properties. It is possible that these domains are subject to regulated proteolysis *in vivo* that affects their localization or activity. GBSSI has distinct catalytic properties (that is, its low specific activity measured *in vitro*), suggesting that it has specific structural features associated with its role in amylose synthesis. Any one plant species probably has several SSs that are active in the soluble phase (Macdonald and Preiss, 1985; Denyer et al., 1995). The extent of their participation in starch synthesis may vary from one species to another and between different parts of the plant. These isoforms may also be bound to the starch granule, perhaps as a result of entrapment as the granule grows (Denyer et al., 1993; Mu et al., 1994). Their activity within the granule may be limited by the highly crystalline conditions of the granule or by the availability of ADPGlucose, whose supply may be severely limiting within the granule.

The relative contributions of different SS isoforms to starch biosynthesis also vary in amount and with time during storage organ development. GBSSI is the major protein bound to starch granules, and it is clear that it is present in large amounts relative to other isoforms (such as GBSSII of pea; Smith, 1990). However, the quantitative contribution of each isoform to  $\alpha(1-4)$ glucan chain production is difficult to assess because of differences in the specific activity of the isoforms and a lack of understanding of the extent to which these differences are present *in vivo*.

There are also differences in the expression of the genes encoding the SS isoforms during storage organ development. Because SSs may become trapped within the granule, where their actual activity is unknown, this is difficult to interpret in terms of relative amounts of each protein functioning in starch biosynthesis. However, GBSSI is maximally expressed later in pea embryo development than is GBSSII (Dry et al., 1992). There is some evidence for a similar differential expression in maize, rice, and potato (Nelson et al., 1978; Dry et al., 1992; Nakamura and Yuki, 1992). The significance of staggered expression of SS isoforms in developing storage organs probably resides in the long-term formation of starch granules. GBSSII synthesis early in organ development would give early amylopectin production. Amylose would increase later due to GBSSI expression, and the relative balance between amylose and amylopectin synthesis would continue to change during development. Perhaps some of the order of the starch granule results from the staggering of the phases of synthesis.

### Production of $\alpha(1-6)$ Branches in Amylopectin

SBE activity is also a function of multiple isoforms. Molecular analysis has identified genes belonging to two SBE families

in maize, rice, and pea (Boyer and Preiss, 1978a, 1978b; Smith, 1988; Smyth, 1988; Fisher et al., 1993; Mizuno et al., 1993; Stinard et al., 1993; Burton et al., 1995), although it is possible that there are other types of SBE in addition to these (Nakamura and Yamanouchi, 1992). The proteins of the two families are structurally related and are similar to glycogen-branching enzymes from bacteria. Members of family A (which comprises SBEII from maize, SBEI from pea, and SBEIII from rice) contain an extra N-terminal domain that is lacking in members of family B (SBEI from maize, SBE from potato, SBEI from rice, SBE from cassava, and SBEII from pea) (Baba et al., 1991; Salehuzzaman et al., 1992; Fisher et al., 1993; Mizuno et al., 1993; Poulsen and Kreiberg, 1993; Burton et al., 1995). This domain is predicted to be flexible, and like the N-terminal arm of GBSSII from pea and potato, it ends in two or three consecutive proline residues (Dry et al., 1992; Burton et al., 1995). The functional significance of this extra domain is as yet unknown, although both SBE isoforms may be located within the starch granule as well as in the soluble phase, as is the case in the pea embryo (Denyer et al., 1993), and this domain might affect the relative partitioning of the enzymes between these two phases. The immunolocalization of the B isoform in potato indicates that it is localized at the interface between the stroma and the starch granule (Kram et al., 1993).

In addition, and perhaps more significantly, the catalytic properties of the isoforms differ (Smith, 1988; Guan and Preiss, 1993; Takeda et al., 1993). Family A isoforms have a lower affinity for amylose than family B members and preferentially use shorter glucan chains during branch formation. The result is that family A members probably make amylopectin with shorter branch lengths than family B isoforms. The most likely cause for this catalytic difference is a conserved structural distinction between the two isoform types. The structure of branching enzymes can be modeled on the structure of  $\alpha$ -amylases, which has been solved by x-ray crystallography (Jespersen et al., 1993). Members of the  $\alpha$ -amylase superfamily, which hydrolyze  $\alpha(1-4)$ -linked glucan chains, consist of a central  $(\beta\alpha)_8$  barrel structure involved in hydrolysis. The SBEs conform to this structure and show conservation of amino acid residues predicted to be involved in hydrolysis, especially in the loops connecting each central  $\beta$ -strand with the following  $\alpha$ -helix (Figure 2; Jespersen et al., 1993; Burton et al., 1995). The loops between  $\beta$ -strand 7 and  $\alpha$ -helix 7 and between  $\beta$ -strand 8 and  $\alpha$ -helix 8 have been implicated in determining the length of branches in comparison with the sequences of glycogen branching enzymes and SBEs. The loop between  $\beta$ -strand 8 and  $\alpha$ -helix 8 is very similar among all family A isoforms and distinct from that in family B isoforms in length and sequence composition, supporting the idea that this loop is involved in the determination of branch length (Burton et al., 1995).

The two SBE isoforms also show distinct developmental differences in the time of their expression. The A isoform is active early in pea embryo development, whereas the activity of the B isoform rises later (Smith, 1988; Burton et al., 1995). This may result in differences in the amylopectin synthesized

early and late in granule formation, because a change from predominantly shorter branch lengths to predominantly longer ones has been detected during pea embryo development (Burton et al., 1995). In other species, the developmental regulation of the SBEs is less significant (Mizuno et al., 1993). This may reflect a less distinct temporal separation of SBE isoform expression in cereal endosperm or stronger developmental gradients within the developing endosperm that may blur temporal differences.

The significance of the contribution of the family A isoform to starch biosynthesis is best appraised through the analysis of mutants; both the *rugosus* (*r*) mutant of pea and *ae* mutants of maize and rice are defective in this activity (Mendel, 1865; Kooistra, 1962; Shannon and Garwood, 1984; Bhattacharyya et al., 1990; Mizuno et al., 1993). Total starch content is reduced by up to 20% in *ae* mutant endosperm and up to 50% in *r* mutant embryos, and the amylopectin content falls from  $\sim 70$  to  $\sim 30\%$ . The remaining starch consists of amylose and an intermediate material that has longer branch lengths than normal amylopectin and a lower molecular weight than amylose. Loss of isoform A activity thus limits the synthesis of starch in a way that cannot be compensated for by isoform B. The inhibition of starch synthesis probably results from a decreasing availability of nonreducing ends in the glucan polymers, which effectively substrate limits SS activity (Edwards et al., 1988). The effect of the loss of isoform A also indicates that it may be responsible for up to 60% of the amylopectin synthesized, although amylopectin synthesis may also be limited indirectly through substrate-limited SS activity in mutant lines. The similarity of the effects of the *ae* and *r* mutations suggests that isoform A plays a similar role in starch synthesis in endosperms and embryos.

There are no known mutants in isoform B activity. This may be of significance in itself. Mutations in starch synthesis are normally identified by reduced starch synthesis during seed formation, which, through the associated increases in sucrose and osmotic potential of the developing embryos, causes a wrinkling or puckering of the dry seed. The fact that no isoform B mutants have been identified suggests that inactivation of this isoform does not limit starch biosynthesis sufficiently to give rise to this phenotype (Müller-Röber and Kossmann, 1994; Burton et al., 1995). Indeed, antisense experiments inhibiting isoform B activity in potato (Kossmann et al., 1991; Müller-Röber and Kossmann, 1994) to undetectable levels resulted in no significant modification in starch synthesis or, indeed, in the amylose-to-amylopectin ratio (Müller-Röber and Kossmann, 1994). Although interpretation of the results is complicated by failure, so far, to identify an isoform A counterpart in potato, the results suggest that isoform B plays a quantitatively minor role in amylopectin biosynthesis in storage organs. It may serve to make amylopectin with somewhat longer distances between the branch points later in granule formation, a qualitative contribution that would be difficult to detect.

Loss of isoform A activity also has a profound effect on starch granule morphology. Granules in *ae* and *r* mutants are small and irregular or deeply fissured (Shannon and Garwood, 1984).

This indicates that the activity of isoform A is essential for the normal regular organization of the starch granule. However, it is not yet clear whether it is the special type of amylopectin synthesized by isoform A that is essential for regular granule formation or rather the relatively early production of amylopectin during granule formation.

An additional enzyme, starch debranching enzyme, may influence the type of glucan produced. The *sugary* (*su1*) mutants of maize, rice, and sorghum have reduced starch levels but contain a highly branched, soluble  $\alpha(1-4)/\alpha(1-6)$ -linked glucan polymer, phytoglycogen (Shannon and Garwood, 1984; Kaushik and Khush, 1991). In *su1* endosperm, starch granules are formed early in development but are subsequently degraded and replaced by phytoglycogen. *su1* mutants are deficient in the activity of debranching enzyme, an  $\alpha(1-6)$ glucosidase (Pan and Nelson, 1984). This supports the hypothesis that the final structure of amylopectin might be determined by a balance between the activities of debranching and branching enzymes. Perhaps debranching enzyme is responsible for reducing the branching in glucan polymers at some stages of storage organ development such that in its absence, the more highly branched phytoglycogen is produced. Clearly, further research in this area is needed. To understand the contribution of debranching enzyme to starch synthesis, it is necessary to ascertain whether the *su1* locus encodes the structural gene for debranching enzyme and the extent to which its effects on other aspects of starch biosynthesis are direct or indirect. It is also important to determine whether debranching activity is significant in the development of starch granules in other species or whether its activity is generally limited to periods of starch mobilization. Any finding of such a contribution of starch debranching enzyme must also be reconciled with the claim that the properties of SBE isoforms can themselves explain the structure of amylopectin (Takeda et al., 1993).

#### THE INTERACTION OF STARCH SYNTHESIS WITH OTHER BIOSYNTHETIC PATHWAYS IN STORAGE ORGAN DEVELOPMENT

Many of the mutations whose primary effect is on starch biosynthesis have pleiotropic effects on other aspects of storage organ development. For example, the *r* mutation of pea confers a wrinkled phenotype on the seed and results in the production of relatively more lipid and less legumin storage protein (summarized in Bhattacharyya et al., 1990). Similarly, starch biosynthetic mutants of maize and barley show reduced production of one class of storage protein, the prolamins. Some of these effects may arise from the increase in sucrose content that results from reduced starch biosynthesis and the consequent increases in osmotic potential (Smith and Denyer, 1992). For example, increases in sucrose cause increased water uptake during development, which stretches the testa or pericarp. However, at maturity, the mutant seed undergoes relatively greater water loss and the overstretched testa or pericarp

becomes wrinkled (Kooistra, 1962). Furthermore, the increase in cell size caused by the increased osmotic potential may result in higher lipid content through increased production of cellular membrane (Coxon and Davies, 1982; Bettey and Smith, 1990).

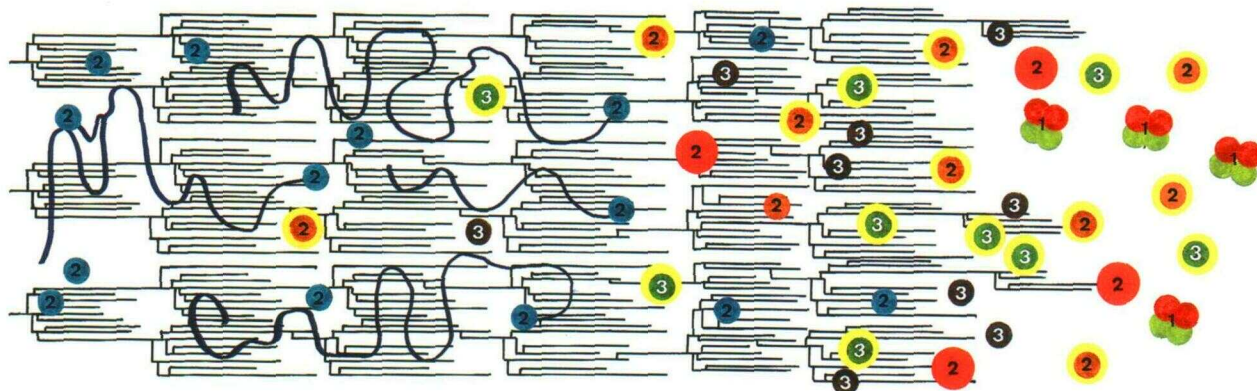
The influence of starch synthesis on storage protein accumulation may also operate through changes in osmotic potential, which may influence the production of particular storage proteins (Turner et al., 1990). It may also involve the coupling of amino acid to sucrose unloading from the phloem supplying the developing storage organs, because if sucrose accumulates in the embryos, unloading from the phloem is likely to be reduced. The resulting limitations in amino acid supply could then limit storage protein synthesis (Bhattacharyya et al., 1993). Whatever the primary cause, limitations on starch biosynthesis may affect both the transcription and translation of specific storage proteins (Turner et al., 1990; Giroux et al., 1994). Inhibition of starch synthesis through antisense inhibition of ADPGPPase activity in potato results in inhibition of patatin production as well and, in addition, affects the overall process of tuber formation, resulting in significantly more tubers per plant (Müller-Röber et al., 1992). These mutants highlight the existence of interactions among the different biosynthetic pathways in storage organ development and in the development of storage organs themselves.

#### CONCLUSIONS

A speculative model for the interaction of the starch biosynthetic enzymes is presented in Figure 3. ADPGPPase (1) in the soluble phase of the amyloplast supplies ADPglucose to SSs (2); those SSs in the soluble phase and to the edge of the granule extend  $\alpha(1-4)$ -linked glucans that are then accessible to the SBEs (3) near the edge of the granule, thus forming amylopectin. These  $\alpha(1-6)$ -branched polymers can then be extended further by SSs. Somewhat protected from the activity of SBEs is amylose, perhaps because the enzyme that makes it, GBSSI, is active deeper within the granule.

This static model cannot explain the growth rings of starch granules, which appear to represent periodic changes in the degree of crystallinity within the starch granule. Nor can it explain the details of the compositional differences across the granule. There is clearly an enormous amount left to understand about the synthesis of starch granules. Central to testing, refining, and developing the model will be the combining of molecular techniques with those of biochemistry and genetics. The enzymes can be produced in *E. coli*, and their catalytic and physical properties can be studied in greater detail. Transgenic plants offer enormous potential for dissecting out the individual contributions of each isoform (Müller-Röber and Kossmann, 1994). In the future, it may be possible to test the model by reconstructing starch biosynthesis in surrogate hosts, such as *E. coli*. Through developing an understanding of how starch is synthesized, the potential for producing novel





**Figure 3.** Speculative Model for the Role of Isoforms in Starch Synthesis within the Starch Granule.

A diagrammatic representation of an amylopectin molecule (black lines) is overlaid with spots representing various isoforms of starch biosynthetic enzymes. Spots labeled 1, ADPGPPase (green spots, small subunit; red spots, large subunit). This enzyme is thought to be active in the soluble phase. Spots labeled 2, SS (blue spots, GBSSI; orange spots, soluble GBSSI; orange spots with yellow ring, GBSSI, which is active in the soluble phase and is also found bound to the granule, where its relative activity *in vivo* is unknown [the yellow ring indicates the N-terminal arm, which can be cleaved without loss of activity]; red spots, other starch synthases that are predominantly soluble but also may be bound to the granule). GBSSI is active within the granule and makes amylose, shown as blue lines, and possibly some amylopectin as well. The other SSSs shown synthesize amylopectin. Spots labeled 3, SBE (green spots with yellow ring, isoform A [yellow ring indicates N-terminal arm]; brown spots, isoform B). Both isoforms are active in the soluble phase and are also found bound to the granule. These isoforms make qualitatively different contributions to amylopectin synthesis.

starches through genetic modification (Shewmaker and Stalker, 1992; Smith and Martin, 1993; Müller-Röber and Kossmann, 1994) may be fully realized.

At present, starch is extracted commercially from a limited number of sources (predominantly maize and potato). Genetic engineering may allow the modification of maize and potato starch toward the quality of starches from other plant sources. In addition, the engineering of starch biosynthesis may lead to modification of starch granule shape, amylose-to-amylopectin ratio, amylopectin chain length, crystallinity, gelling properties, phosphorylation, and lipid content—all of which will alter significantly the technological properties of starch for use in both food and nonfood industries.

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